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A PRACTICAL GUIDE TO MOLECULAR CLONING

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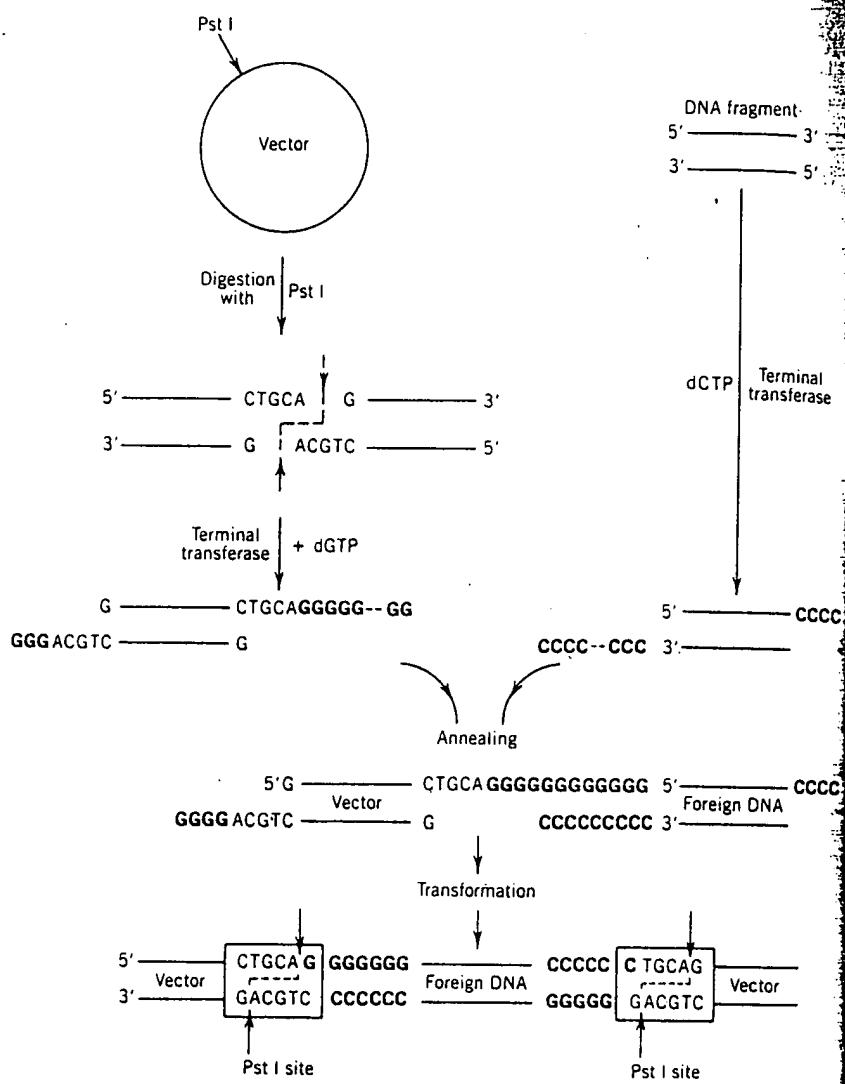


Figure 63. Cloning of a DNA fragment with dC tails into a vector whose cloning site has been elongated with dG tails. The method shown allows the recovery of the cloned foreign DNA because the Pst I recognition sites are regenerated after ligation.

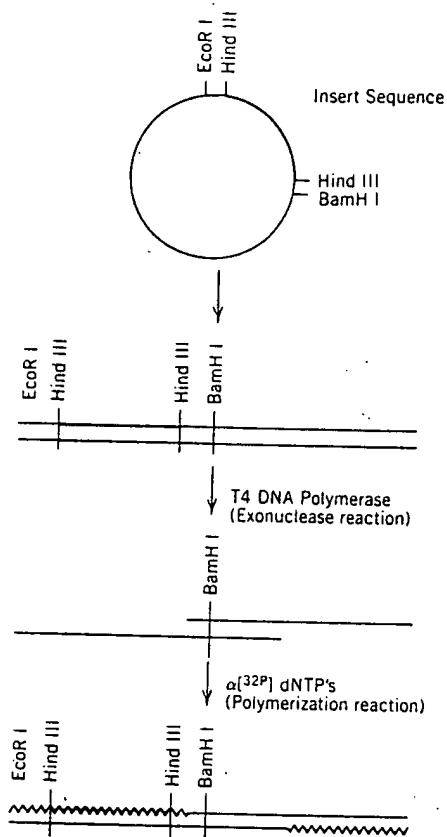


Figure 74. Selective labeling with T4 DNA polymerase of individual strands of a 1-kb insert within the Hind III site of pBR322.

0.5 mM DTT

Store as frozen aliquots of a 10× buffer.

Small variations in the buffer composition have no detectable effect on the reactions. The following experimental protocols take advantage of this to simplify the reaction mixtures.

EXAMPLE 1: PREPARATION OF A HYBRIDIZATION PROBE

For this example consider a 5-kb hybrid plasmid with a single Hind III site adjacent to a 1-kb inserted fragment. The object of this protocol is to prepare a hybridization probe for detection of the sequences present on the insert.

SYNTHESIS OF cDNA 423

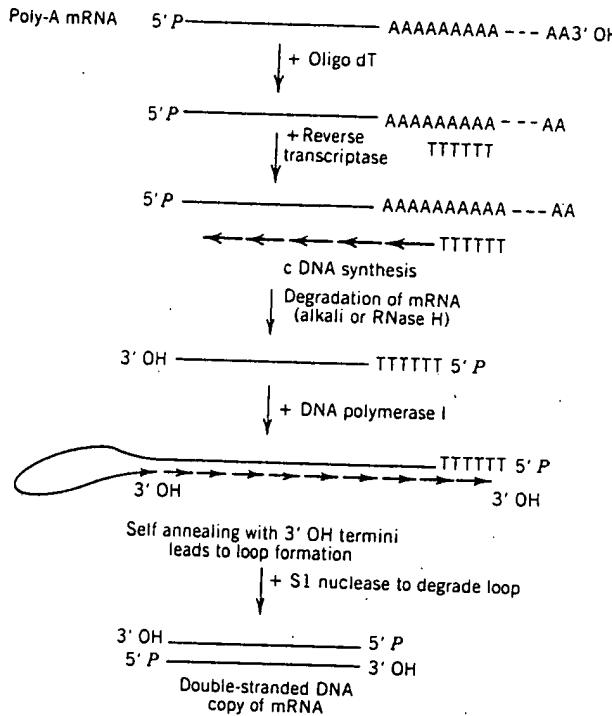


Figure 93. Synthesis of cDNA from poly mRNA.

7. Cool on ice and spin for 2 seconds at 12,000 rpm.

2. Synthesis of the Second Strand

8. Add:

- 5.2 μ l sterile distilled water
- 2.0 μ l 1 M HEPES-KOH (pH 6.9)
- 1.6 μ l 1 M KCl
- 0.6 μ l 0.1 M $MgCl_2$
- 0.4 μ l 0.1 M DTT
- 0.2 μ l 10 mM dNTP

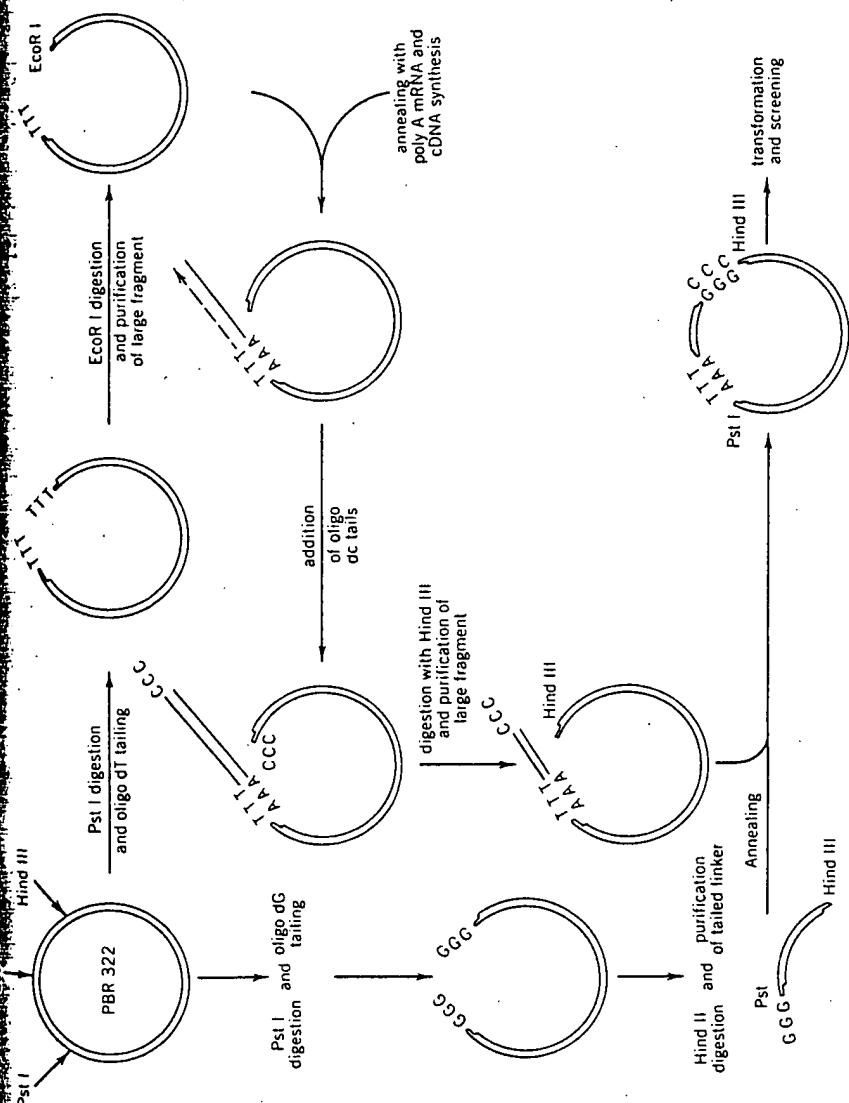


Figure 94. Direct cloning of poly-A RNA species in pBR322. [After Okayama and Berg (1)].

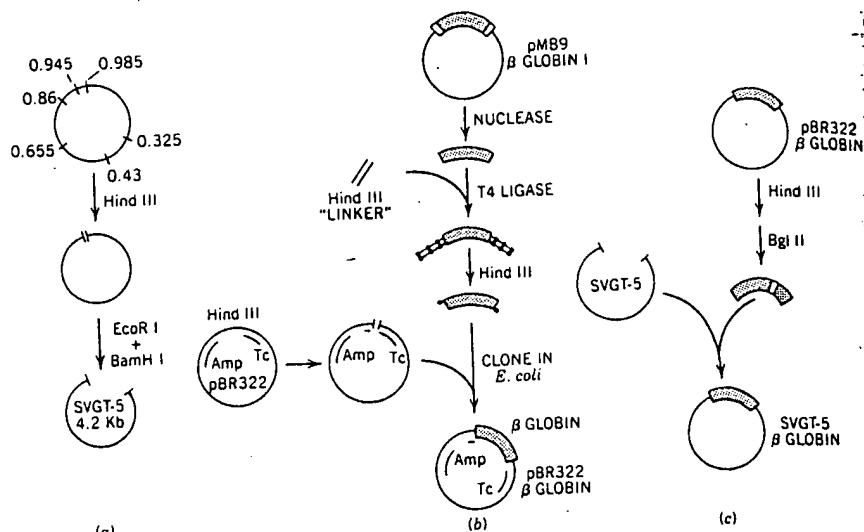


Figure 113. Scheme for construction of the SV40- β G recombinant genome. (a) Construction of SVGT5 vector. (b) Subcloning of β -globin cDNA. (c) Cloning of β -globin cDNA into SVGT5. Reprinted by permission from *Nature*, 277, 109. Copyright 1979 Macmillan Journals Limited. [With permission of P. Berg. Copyright (1979) Macmillan Press.]

such amplicons are used to transfect eucaryotic cells (in the presence of helper virus DNA) full-length chimeric defective genomes of approximately 150 kb are generated. They consist of multiple head-to-tail reiterations of the inserted cloned amplicon, which can be introduced back into bacteria, providing a very convenient shuttle cloning system (Figure 118, page 507).

A 12-kb DNA fragment containing the chicken ovalbumin gene with its 5' and 3' flanking sequences and an 11.7-kb fragment containing the gene coding for the α subunit of human chorionic gonadotropin have been cloned in the HSV amplicon and stably propagated in virus stocks (Figure 119, page 508).

It is not yet clear if the promoters for eucaryotic genes inserted into defective genomes will be efficiently active in infected cells. However, there is strong evidence suggesting that repeat-unit DNA is preferentially transcribed in infected cells and that more abundant expression of the corresponding gene takes place under these conditions.

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Yeast Vectors

1. See: "Cloning of Genes into Yeast Cells," in *Methods in Enzymology*, Vol. 101, R. Wu, L. Grossman, and K. Moldave, Eds., Academic Press, New York (1983), pp. 167-343.

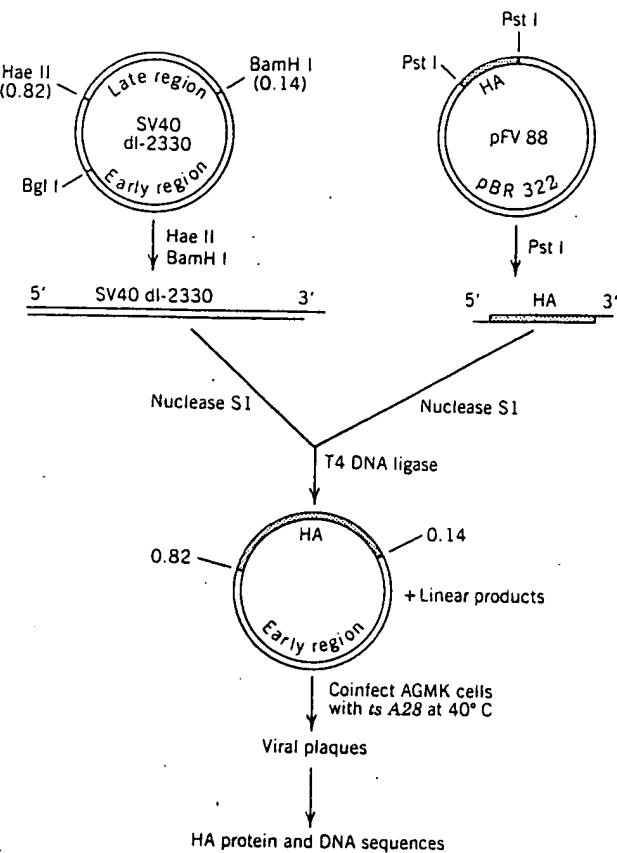


Figure 117. Construction and isolation of a hemagglutinin (HA)-SV40 hybrid virus. (With permission of C. J. Lai.)

19. Stow, N. D., and McMonagle, E. C., in *Eucaryotic Viral Vectors*, Y. Gluzman, Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982), p. 199.

For a General Review

20. Gluzman, Y., Ed., *Eucaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

3. TRANSFECTION OF EUKARYOTIC CELLS WITH PURIFIED DNA

Manipulated DNA cannot always be reintroduced into eucaryotic cells by means of nonlytic or nontransforming viral vectors. Techniques have been